

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Szyperski et al.)	Examiner:
)	Y. Gakh
Serial No.	: 10/628,818)	
)	Art Unit:
Cnfrm. No.	: 5870)	1743
)	
Filed	: July 28, 2003)	
)	
For	: PHASE SENSITIVELY-DETECTED REDUCED)	
	DIMENSIONALITY NUCLEAR MAGNETIC)	
	RESONANCE SPECTROSCOPY FOR RAPID)	
	CHEMICAL SHIFT ASSIGNMENT AND)	
	SECONDARY STRUCTURE DETERMINATION OF)	
	PROTEINS)	

DECLARATION OF THOMAS A. SZYPERSKI UNDER 37 C.F.R. § 1.132

Mail Stop:

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Thomas A. Szyperski, pursuant to 37 C.F.R. § 1.132, declare:

1. I received a Diploma degree in Chemistry from Technical University of Munich, Germany in 1988 and a Dr. Sc. degree in Chemistry from ETH Zurich, Switzerland in 1992.
2. I am currently Professor of Chemistry and Biochemistry, and Director of the UB High-Field NMR Facility at University at Buffalo, The State University of New York, Buffalo, New York. I am also currently Adjunct Senior Researcher at the Hauptman-Woodward Medical Research Institute, Buffalo, New York.
3. As indicated in my attached Curriculum Vitae (Exhibit 1) and list of publications (Exhibit 2), I have authored or co-authored over 100 mostly peer-reviewed professional publications in the fields of nuclear magnetic resonance (NMR) techniques and

structure determination of biological macromolecules using NMR spectroscopy. Since 1999, I have given over 120 invited lectures in these same technical fields (see Exhibit 1).

4. I am an elected member of the American Chemical Society, American Association for the Advancement of Science, and the Gesellschaft Deutscher Chemiker.

5. I am an inventor of the above-identified patent application.

I am presenting this declaration to demonstrate that, contrary to the statement on page 2 of the outstanding office action for the above-identified patent application, none of the NMR experiments disclosed or claimed in U.S. Patent No. 7,141,432 to Szyperski ("Szyperski") disclose or suggest conducting four reduced dimensionality (RD) nuclear magnetic resonance (NMR) experiments on the protein sample, wherein the chemical shift values of ^1H and ^{13}C which are encoded in peak pairs of an NMR spectrum are detected in a phase sensitive manner (emphasis added).

6. I am familiar with the claims and disclosure of Szyperski.

7. Claims 1-40 of Szyperski are drawn to a method for obtaining rapid and complete assignments of chemical shift values of ^1H , ^{13}C and ^{15}N of a protein molecule comprising: providing a $^{15}\text{N}/^{13}\text{C}$ -labeled protein sample; and conducting four RD NMR experiments on the protein sample, where (1) a first experiment is selected from the group consisting of a RD 3D $\text{H}^{\alpha/\beta}\text{C}^{\alpha/\beta}(\text{CO})\text{NHN}$ NMR experiment, a RD 3D $\text{HA,CA,}(\text{CO}),\text{N,HN}$ NMR experiment, and a RD 3D $\text{H,C,}(\text{C-TOCSY-CO}),\text{N,HN}$ NMR experiment for obtaining sequential correlations of chemical shift values; (2) a second experiment is selected from the group consisting of a RD 3D HNNCAHA NMR experiment, a RD 3D $\text{H}^{\alpha/\beta}\text{C}^{\alpha/\beta},\text{N,HN}$ NMR experiment, and a RD 3D $\text{HNN}<\text{CO,CA}>$ NMR experiment for obtaining intraresidue correlations of chemical shift values; (3) a third experiment is a RD 3D H,C,C,H-COSY NMR experiment for obtaining assignments of sidechain chemical shift values; and (4) a fourth experiment is a RD two-dimensional (2D) $\text{HB,CB,}(\text{CG,CD}),\text{HD}$ NMR experiment for obtaining assignments of aromatic sidechain chemical shift values.

8. The claims of Szyperski neither disclose nor suggest detection of chemical shift values of ^1H and ^{13}C which are encoded in peak pairs of an NMR spectrum in a phase sensitive manner, as required by the claims of the present application (emphasis added).

More specifically, in Szyperski, chemical shifts (also referred to as the ‘projected chemical shifts’) inferred in RD NMR experiments from the frequency difference of two peaks forming a peak pair arise from cosine modulation (*see, e.g.*, Szyperski at column 4, lines 12-24, column 4, lines 44-55, col. 5, lines 8-22, col. 5, lines 44-57, col. 6, lines 7-18, col. 6, lines 36-47, col. 6, line 65 to col. 7, line 8, and col. 7, lines 23-33). Chemical shifts which are detected using cosine modulation are *not* measured in a phase-sensitive manner. That is, in Szyperski, the frequency difference can relate to a chemical shift being either downfield or up-field to the carrier frequency of the radio-frequency pulse exciting the corresponding spins. In RD NMR, as described in Szyperski, this ambiguity must be resolved by placing the carrier at the edge of the spectral range so that *all* frequency differences correspond to chemical shifts being either downfield or up-field from the carrier frequency. This, however, requires that the spectral width and thus the number of increments that are required to reach a given maximal evolution time has to be increased. In turn, the minimal measurement time is increased by the same amount.

9. Szyperski does teach the generation of certain NMR signals encoding the chemical shift values of $^{13}\text{C}_{i-1}^{\alpha}$ and $^{15}\text{N}_i$ (col. 4, lines 8-9), $^{13}\text{C}_{i-1}^{\text{ali}}$ and $^{15}\text{N}_i$ (col. 4, lines 40-42), $^{13}\text{C}_i^{\alpha}$, $^{13}\text{C}_i^{\beta}$, and $^{13}\text{C}_i^{\gamma}$ (col. 5, lines 4-5), $^{13}\text{C}_i^{\alpha}$, $^{13}\text{C}_i^{\beta}$, and $^{15}\text{N}_i$ (col. 5, lines 39-41), $^{13}\text{C}^m$ and $^{13}\text{C}^n$ (col. 6, lines 3-5 and 32-33), $^{13}\text{C}^{\beta}$ (col. 6, lines 61-63), and $^{13}\text{C}^m$ (col. 7, lines 20-21) in a phase sensitive manner. However, this does not relate to detection of chemical shift values of ^1H and ^{13}C which are encoded in peak pairs of an NMR spectrum in a phase sensitive manner, as required by the claims of the present invention (emphasis added). In contrast, all teachings in Szyperski relating to chemical shifts of ^{13}C and ^1H inferred in RD NMR experiments from the frequency difference of two peaks forming a peak pair arise from cosine modulation. As described above, chemical shifts arising from cosine modulation are not measured in a phase sensitive manner. Szyperski neither attempts to nor achieves the determination of chemical shift values of ^1H and ^{13}C which are encoded in peak pairs of an NMR spectrum in a phase sensitive manner.

10. This key drawback of RD NMR as disclosed in Szyperski is resolved by introducing the sine modulation as described in the present patent application. The sine modulation results in an anti-phase peak pair in which either the up-field or the down-field peak is of positive sign (while the other peak of the peak pair is of negative sign). Hence, the

distinction of a peak pair with 'positive peak up-field and negative peak down-field' from a peak pair with 'negative peak up-field and positive peak down-field' enables phase sensitive detection of the chemical shift inferred from the separation of the peaks of the peak pairs generated by sine modulation. The experiments disclosed in the present application represent the fastest possible way to phase-sensitively detect projected chemical shifts encoded in a frequency separation of two peaks.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 12/6/2007

Thomas Szyperski
Thomas A. Szyperski

EXHIBIT 1

Curriculum Vitae

Dr. sc. nat ETH Thomas Szyperski

Professor

Director, UB and Roswell Park Cancer Institute NMR facilities

The State University of New York at Buffalo

Chemistry Department

(‘cross appointed’ in Departments of Biochemistry and Structural Biology)

816 Natural Sciences Complex

Buffalo, NY 14260, USA

Date of birth	December 13, 1963
Place of birth	Berlin, Germany
Nationality	German
Obligatory Military Service	July 1 1982 - September 30, 1983

Education

Vordiplom Biochemistry, University of Tübingen, Germany	1984
Vordiplom Chemistry, Technical University München, Germany	1985
Diploma Chemistry, Technical University München, Germany	1988
Dr. sc. nat. ETH, ETH Zürich, Switzerland	1992
Mentor: Prof. K. Wüthrich	
Habilitation, ETH Zürich, Switzerland	1998

Employment History

TU München, Germany	3.1988 - 9.1988
University of Auckland, New Zealand	10.1988 - 2.1989
ETH Zürich, Switzerland	3.1989 - 10.1998
State University of New York, USA	11.1998 –

Other Positions

Member of

Operating Committee of the New York Structural Biology Center
(<http://www.nysbc.org/>)

Executive Committee of the Northeast Structural Genomics Consortium
(<http://www.nesg.org>)

Leader of

NMR Division of the Northeast Structural Genomics Consortium
(<http://www.nesg.org/>)

Awards

- | | |
|-----------------|---|
| 7.1981 / 7.1982 | Member of the german national team participating at the XIII. and XIV. International Chemistry Olympiads - awarded Silver and Golden Medal, respectively. |
| 5.1993 | 'Medaille der ETH' for outstanding dissertations |
| 7.1998 | 'Privatdozent' ETH Zürich |
| 11.1999 | Research Innovation Award of the Research Corporation |
| 3.2003 | Buck-Whitney Medal of the American Chemical Society |
| 11.2003 | <i>Scientific American</i> 50 Award for 'Chemistry and Material Sciences' |
| 4.2006 | Laukien Prize 2006 |
| 5.2007 | SUNY Chancellor's Award for Excellence in Scholarship and Creative Activities |

Honors

- | | |
|-----------------|---|
| 9.1982 - 9.1988 | Scholar of the 'Studienstiftung des Deutschen Volkes' |
| 6.1989 - 9.1991 | Scholar of the 'Verband der Chemischen Industrie' |
| 5.1999 - | Adjoined Senior Researcher at 'Hauptman-Woodward Medical Institute' |

Professional Memberships and Activities

Member of

American Chemical Society

Gesellschaft Deutscher Chemiker

American Association for the Advancement of Science

Member of

Faculty of 1000

Professional Service

Member of

Editorial board of the 'Journal of Structural and Functional Genomics'

Reviewer for scientific journals:

Applied and Environmental Microbiology

Biochemistry

Biopolymers

Bioinformatics Journal

Biotechnology and Bioengineering

Biotechnology Progress

BioTechniques

European Journal of Biochemistry

Journal of the American Chemical Society

Journal of Bacteriology

Journal of Biomolecular NMR

Journal of Magnetic Resonance

Journal of Molecular Biology

Journal of Structural and Functional Genomics

Macromolecules

Magnetic Resonance in Chemistry

Metabolic Engineering

Nature Biotechnology

Structure

Community Service

Reviewer for

NIH: BBCA study section (2000)

NIH: S10 Instrumentation Panel (2004)

NIH: NCI site review (2006)

NSF: Major Research Instrumentation Panel (2001)

NSF: *Ad Hoc* Reviewer (2002 - 2005)

NSF: Panel Member (2004, 2005, 2006, 2007)

Wellcome Trust, United Kingdom (2001; 2003)

Genome Canada (2004)

Wiener Wirtschafts-, Forschungs- und Technologiefonds (2005)

Israel Science Foundation (2005)

U.S. Patents

Method of using G-matrix Fourier transformation nuclear magnetic resonance (GFT NMR) spectroscopy for rapid chemical shift assignment and secondary structure determination of proteins.
US patent number 6.831.459.

Method of using Reduced Dimensionality nuclear magnetic resonance spectroscopy for rapid chemical shift assignment and secondary structure determination of proteins.
US patent number 7.141.432

Invited Lectures (1999 -)

1.USA

- (1) "NMR Spectroscopy: a Powerful Tool for Life Scientists"
Biochemistry Department, State University of New York at Buffalo
Buffalo, New York, February 15, 1999.
- (2) "Sequential Resonance Assignment of Medium-sized $^{15}\text{N}/^{13}\text{C}$ -Labeled Proteins with Projected 4D Triple Resonance Experiments"
Varian NMR Users Conference
Orlando, Florida, March 1, 1999.
- (3) "Indirect Detection of ^{13}C using 1D and 2D [^{13}C , ^1H]-correlation NMR Spectroscopy.
NIH Symposium: ^{13}C in Metabolic Research, University of Texas Medical Branch
Dallas, Texas, May 6, 1999.
- (4) "NMR Spectroscopy in Structural Biology"
Center of Advanced Research in Molecular Biology and Immunology
Buffalo, New York, May 18, 1999.
- (5) "NMR Spectroscopy Applied in Structural Biology and Metabolic Research"
Hauptman-Woodward Medical Research Institute
Buffalo, New York, June 10, 1999.
- (6) "NMR at SUNYAB"
1st Upstate New York NMR Symposium, Wadsworth Center
Albany, New York, October 4, 1999.
- (7) "Reduced Dimensionality NMR Experiments for Structural Genomics",
Symposium NE Structural Genomics Consortium Project Team, Rutgers University,
Piscataway, New Jersey, November 2, 1999.
- (8) "Structural Biology of the Mitochondrial Origin of Light Strand DNA Replication" Rockefeller
University, New York, New York, December 13, 1999.
- (9) "Reduced Dimensionality NMR Spectroscopy for Structural Genomics"
Northeastern Structural Genomics Consortium, Rutgers University,
Piscataway, New Jersey, December 14, 1999.
- (10) "Novel RD NMR Experiments for Structural Genomics"
NESG Consortium Workshop,
Princeton, New Jersey, May 13, 2000.
- (11) "METAFoR by NMR: an Approach Comes of Age"
Cargill Dow Polymers,
Minnetonka, Minneapolis, May 24, 2000.

- (12) "Reduced-dimensionality NMR for Structural Genomics"
Pacific Northwest National Laboratories,
Redland, Washington, July 29, 2000.
- (13) "Structural Biology in Supercooled Water"
2nd Upstate New York NMR Symposium, Cornell University,
Ithaca, New York, October 2, 2000.
- (14) "News on RD NMR"
Cornell Medical School
New York, New York, November 28, 2000.
- (15) "Reduced-dimensionality NMR spectroscopy"
RD NMR workshop UB high-field NMR facility and CCR Buffalo,
Buffalo, New York, December 5, 2000.
- (16) "Structural Genomics by NMR"
Foster Chemistry Colloquia UB Chemistry Department
Buffalo, New York, December 8, 2000.
- (17) "Reduced-dimensionality NMR Spectroscopy: An Approach Comes of Age"
Keystone Symposia, Frontiers of NMR in Molecular Biology
Big Sky, Montana, January 22, 2001.
- (18) "Bio-NMR at UB: Supercool!"
Chemistry Department, Youngstown State University
Youngstown, Ohio, February 9, 2001.
- (19) "Structural Biology in Supercooled Water"
UB Physics Department Seminar Series
Buffalo, New York, February 13, 2001.
- (20) "Structural Biology in Supercooled Water"
Varian NMR Users Conference
Orlando, Florida, March 9, 2001.
- (21) "Structural Genomics by NMR"
Center for Computational Research 2000-2001 Colloquium Series
Buffalo, New York, March 27, 2001.
- (22) "Metabolic Flux Ratio and Bioreaction Network Topology Analysis by NMR"
Department of Plant Biology Michigan State University
East Lansing, Michigan, April 25, 2001.
- (23) "Metabolic Flux Ratio and Bioreaction Network Topology Analysis by NMR"
Microbia Inc.
Boston, Massachusetts, April 27, 2001.

- (24) "Structural Biology in Supercooled Water"
3rd Upstate New York NMR meeting
Rochester, New York, October 15, 2001.
- (25) "Metabolic Profiling by NMR"
Industrial Associates Program New Jersey
Princeton, New Jersey, October 16, 2001.
- (26) "RD NMR for Structural Genomics"
Bristol-Myers-Squibb
Princeton, New Jersey, October 17, 2001.
- (27) "Structural Biology in Supercooled Water"
NJ American Chemical Society NMR Topic Group
Princeton, New Jersey, October 17, 2001.
- (28) "Metabolic Profiling by NMR" Metabolic Profiling: Pathways in Discovery Cambridge
Healthtech Institute's Premier Conference
Chapel Hill, North Carolina, December 4, 2001.
- (29) "Structural Genomics by NMR"
NIEHS
Chapel Hill, North Carolina, December 5, 2001.
- (30) "NMR at UB"
Kent State University
Kent, Ohio, January 31, 2002.
- (31) "NMR Sample Preparation for High-throughput Structure Determination"
NIH-PSI workshop
Bethesda, Maryland, March 7, 2002.
- (32) "NMR-based structural genomics"
Buffalo-Niagara Post-genomic Research Conference
Buffalo, New York, May 15, 2002.
- (33) "New NMR methods for Structural Genomics"
NESG consortium, Annual Meeting Center for Advanced Biotechnology and Medicine
Piscataway, New Jersey, June 10, 2002.
- (34) "News on RD NMR"
NIH workshop University of Wisconsin
Madison, Wisconsin, June 21, 2002.
- (35) "Metabolic Profiling: New Insights"
2002 Annual Meeting of the Society for Industrial Microbiology
Philadelphia, Pennsylvania, August 11-15, 2002.

- (36) "NMR Methodology for High-Throughput Protein Resonance Assignment"
Biotechnology Forum
Buffalo, New York, October 22, 2002.
- (37) "NMR in the post-genomic era"
Juniata College
Huntingdon, Pennsylvania, November 19, 2002.
- (38) "GFT NMR Spectroscopy"
Rutgers University
Piscataway, New Jersey, December 11, 2002.
- (39) 'NMR spectroscopy for structural genomics'
Rutgers University
Piscataway, New Jersey, January 22, 2002.
- (40) 'GFT NMR, a new approach to rapidly obtain precise high-dimensional NMR spectral information'
Keystone Symposium, Frontiers in Structural Biology
Taos, New Mexico, February 8, 2003.
- (41) 'NMR methodology for structural genomics'
Rutgers University
Piscataway, New Jersey, February 13, 2003.
- (42) 'NMR in the postgenomic era'
Roswell Park Cancer Institute
Buffalo, New York, February 24, 2003.
- (43) 'NMR-based Structural Genomics: New Methods and Perspectives'
New York State Proteomics Symposium
Syracuse, New York, March 17, 2003.
- (44) 'GFT NMR: rapid and precise NMR data collection'
Varian Users Meeting
Savannah, Georgia, March 29, 2003.
- (45) 'Implementation of GFT NMR experiments'
Bruker Users Meeting
Savannah, Georgia, March 29, 2003.
- (46) 'GFT NMR Spectroscopy: Theory and Applications'
44th Experimental NMR Conference
Savannah, Georgia, April 4, 2003.
- (47) 'NMR Methodology for Structural Genomics'
Buffalo Excellence in Biological Sciences Seminar Series
Buffalo, New York, May 15, 2003.

- (48) 'RD and GFT NMR: new NMR methods for rapid protein structure determination'
Middle Atlantic Regional Meeting of the ACS
Princeton, New York, June 11, 2003.
- (49) 'NMR for structural biology and metabolic profiling'
Northeastern Regional Meeting of the ACS
Saratoga Springs, New York, June 18, 2003.
- (50) 'Profiling Yeast Metabolism by NMR'
National meeting of the American Chemical Society
New York, New York, September 12, 2003.
- (51) 'NMR at UB: Structural Genomics and Metabolic Flux Profiling'
Seminar Series
Roswell Park Cancer Institute
Buffalo, New York, February 20, 2004
- (52) 'GFT NMR News'
45th Experimental NMR Conference,
Asilomar, California, April 23, 2004.
- (53) "NMR Methods Enabling Rapid Data Collection"
EMSL meeting 2004, Pacific Northwest National Laboratories
Redland, Washington, June 16, 2004.
- (54) 'Rapid Acquisition of Multidimensional NMR data'
Gordon Research Conference in Stereochemistry
Salve Regina University
Newport, Rhode Island, June 21, 2004
- (55) 'GFT NMR – Toward HTP NMR Structure Determination'
Departmental Lecture Series, Chemistry Department
University of Rochester
Rochester, New York, October 27, 2004
- (56) 'Rapid Sampling of NMR Data'
International Conference on Structural Genomics
Washington, DC, November 20, 2004
- (57) 'NMR Methodology for Structural Genomics'
NESG Methodology Retreat
Rutgers University
New Brunswick, NJ, March 13, 2005
- (58) 'Fast Acquisition of Multidimensional NMR Data: Implications for Structural Genomics'
Departmental Lecture Series, Chemistry Department
Fayetteville, Arkansas, March 28, 2005

- (59) 'GFT NMR – Rapid Protein NMR Data Collection for Structural Genomics'
Departmental Lecture Series, Chemistry Department
Seattle, Washington, March 30, 2005
- (60) 'New Methodology for PSI-2: GFT NOESY and G2FT NMR'
NESG NMR Division Workshop
Buffalo, New York, May 24, 2005
- (61) 'Where do we stand on HTP NMR Structure Determination'
NESG Retreat
Princeton, New Jersey, June 21, 2005
- (62) 'GFT NMR-based Structural Genomics'
Keystone Symposia, Frontiers in Structural Biology
Keystone, Colorado, January 31, 2006
- (63) 'High-throughput Protein Structure Determination by NMR: New Methodology & Impact on Structural Biology'
Amgen Inc.
Thousand Oaks, California, March 17, 2006
- (64) 'GFT Projection NMR Spectroscopy: Principles and Applications'
Amgen Inc.
Thousand Oaks, California, March 17, 2006
- (65) 'GFT NMR based Structural Biology'
Departmental Lecture Series, Physics Department
Washington University
St. Louis, Missouri, April 3, 2006
- (66) 'G-matrix Fourier Transform Projection NMR Spectroscopy'
Laukien Prize Lecture
Experimental NMR Conference
Asilomar, California, April 24, 2006
- (67) 'GFT Projection NMR'
Cleveland Center for Structural Biology NMR Symposium
Cleveland, Ohio, May 13, 2006
- (68) 'G-matrix Fourier Transform Projection NMR: Theory and Application'
Workshop on 'NMR data Collection and Analysis'
University of Wisconsin
Madison, WI, June 6, 2006
- (69) 'NMR-based structural genomics'
Canisius College, Chemistry Department
Buffalo, NY, September 8, 2006

- (70) 'Methodology for rapid NMR data collection'
Eastern Analytical Session
Somerset, NJ, November 13, 2006
- (71) 'GFT Projection NMR: Application in Structural Biology'
Departmental lecture series, Chemistry Department
University of Illinois at Urbana-Champaign
Urbana-Champaign, IL, November 29, 2006
- (72) 'Structural Genomics in an 'omics'-world'
Metabonomics Research Day,
University at Buffalo,
Buffalo, NY, January 8, 2007
- (73) 'GFT NMR for membrane proteins'
Retreat, New York Center on Membrane Protein Structure
New York Structural Biology Center,
New York, NY, March 14, 2007
- (74) 'Role of GFT Projection NMR for NESG'
CCPN workshop
Rutgers University
New Brunswick, NJ, March 15, 2007
- (75) 'Structural Genomics in an 'omics'-world'
Seminar Series
Department of Gynecology
University at Buffalo, April 25, 2007
- (76) 'The NESG NMR Program'
NESGC NIH site visit
Rutgers University
New Brunswick, NJ, May 4, 2007
- (77) 'GFT Projection NMR Spectroscopy'
SECNMR
Web-seminar, September 26, 2007
- (78) 'Where do we stand for Projection NMR spectroscopy for HTP protein structure determination?'
Amgen Inc.
Thousand Oaks, California, October 6, 2007
- (79) 'Where do we stand on Projection NMR Spectroscopy'
Upstate NY NMR Symposium
SUNY College of ESC
Syracuse, NY, October 12, 2007

(80) 'NMR can be entertaining'

New Jersey NMR topical group

Somerset, NJ, November 12, 2007

(81) 'GFT NMR Spectroscopy: application for membrane protein structure determination'

New York Structural Biology Center

New York, NY, November 30, 2007

2. International

- (1) "Synergy of ^{13}C -labeling of Amino Acids and Metabolic Flux Balancing- a Novel Approach to Support Process Design in Biotechnology"
International Conference on Magnetic Resonance in Biological Systems,
Tokyo, Japan, August 27, 1998.
- (2) "Exploration of Central Carbon Metabolism using Biosynthetic Fractional ^{13}C -Labeling and Two-dimensional NMR Spectroscopy"
Metabolic Engineering Conference II
Elmau, Germany, October 16, 1998.
- (3) "METAFor by NMR Analysis for Biotechnology Research"
ETH Zürich
Zürich, Switzerland, September 2, 1999.
- (4) "NMR Structure of a Chimeric Hybrid Duplex Formed During Initiation of HIV-1 Reverse Transcription"
4th Annual Workshop "Structure-Function Analysis of Drug Resistant HIV-RT"
Rome, Italy, November 12, 1999.
- (5) "Neuere Erkenntnisse über lebende Systeme mittels NMR Spektroskopie"
Universität Düsseldorf
Düsseldorf, Germany, January 10, 2000.
- (6) " ^{13}C -labeling Experiments in Support of Biotechnology Research"
Swiss National Science Foundation Symposium: The Swiss Priority Project Biotechnology,
ETH Zürich,
Zürich, Switzerland, March 23, 2000.
- (7) "Towards Structural Biology in Supercooled Water. Implications for the Structure of FluA"
Technische Universität München
München, Germany, March 29, 2000.
- (8) "Kernresonanzspektroskopie - faszinierende neue Möglichkeiten zur Ergründung biomolekularer Vorgänge"
Universität Siegen
Siegen, Germany, July 4, 2000.
- (9) "Reduced Dimensionality NMR for Structural Genomics"
Protein Engineering Network of Centers of Excellence
Toronto, Canada, October 19, 2000.
- (10) "Rapid NMR assignment of Proteins for High-throughput Structure Determination"
1st International Conference on Structural Genomics (ICSG) 2000
Yokohama, Japan, November 3, 2000.
- (11) "Structural Biology in Supercooled Water"
NMR in Molecular Biology, European Science Foundation (ESF)
Karrebaksminde, Denmark, June 11, 2001.

(12) “Structural Genomics by NMR”

Institute of Biotechnology

Vilnius, Lithuania, October 5, 2001.

(13) “Structural Biology in Supercooled Water”

Institute of Biotechnology

Vilnius, Lithuania, August 6, 2002.

(14) “Structural Biology in Supercooled Water”

20th International Conference on MR in Biological Systems

Toronto, Canada, August 27, 2002.

(15) “Flux Information from NMR Data”

FEBS Course “Advanced Technologies For Metabolic Engineering in Biotechnology and Medicine”

Lisbon, Portugal, September 7-14, 2002.

(16) “NMR for Metabolic Profiling: New Insights”

FEBS Course “Advanced Technologies For Metabolic Engineering in Biotechnology and Medicine”

Lisbon, Portugal, September 7-14, 2002.

(17) ‘GFT NMR’

Biochemistry Department, University of Toronto

Toronto, Canada, February 27, 2003.

(18) ‘NMR in high-throughput: Structural Genomics and Metabolic Flux Profiling’

AstraZeneca Biotechnology Seminar Series

Mississauga, Canada, February 27, 2003.

(19) ‘Strukturelle Genomik: Semiempirische Lösung des Proteinfaltungsproblems?’

Seminar Series of the ‘Bayreuther Zentrum für Molekulare Biowissenschaften’

Bayreuth, Germany, April 25, 2003.

(20) ‘GFT NMR spectroscopy: Rethinking Multidimensional Data Acquisition’

16th International Conference on NMR Spectroscopy

Cambridge, UK, July 1, 2003.

(21) ‘GFT NMR for rapid NMR data acquisition’

Jahrestagung der Fachgruppe Resonanzspektroskopie (GDCh)

Leipzig, Germany, October 2, 2003.

(22) ‘Structural Genomics by NMR: Novel Methods and Insights’

3rd NCCR Symposium on New Trends in Structural Biology

Switzerland, Zürich, November 15, 2003.

(23) ‘GFT NMR, Progress for Rapid NMR Data Collection’

21st International Conference on Magnetic Resonance in Biological Systems

Hyderabad, India, January 2005

- (24) 'GFT NMR Based Protein Structure Determination in High-Throughput'
Keystone Symposium, Frontiers in Structural Biology
Banff, Canada, February 1, 2005
- (25) 'Strukturelle Genomik: Revolution in Grundlagenforschung und
Medikamentenentwicklung'
Virtoweb, Support of IT for Biotech
Bochum, Germany, February 18, 2005
- (26) 'Studies of the M.HhaI – DNA system'
Institute for Biotechnology
Vilnius, Lithuania, August 18, 2005
- (27) 'Protocol for NMR-based Structural Proteomics'
HUPO 4th Annual World Congress
Munich, Germany, August 29, 2005
- (28) 'NMR-based Structural Genomics'
Chemistry Department Lecture Series
Reykjavik, Iceland, October 7, 2005
- (29) 'Methodology for NMR-based Structural Genomics'
NMR Department, Max-Planck Institute for Biophysical Chemistry
Göttingen, Germany, October 24, 2005
- (30) 'GFT Projection NMR Spectroscopy'
University of Halle, Physics Lecture Series
Halle, Germany, October 27, 2005
- (31) 'NMR for Structural Genomics'
SCAI of the Fraunhofer Gesellschaft
Bonn, Germany, November 9, 2005
- (32) 'GFT NMR for structural and dynamic studies of proteins in solution '
7th Igler NMR-symposium
Oberurgl, Austria, February 14, 2006
- (33) 'NMR studies of M.HhaI-DNA complexes'
Institute for Biotechnology
Vilnius, Lithuania, May 29, 2006
- (34) 'High-throughput protein structure determination for NMR-based structural genomics'
RWTH Aachen, Chemistry Department
Aachen, Germany, September 28, 2006
- (35) 'G-matrix Fourier Transform projection NMR spectroscopy'
RIKEN NMR Center
Yokohama, Japan, October 21, 2006

(36) 'GFT NMR data acquisition, processing and analysis'
RIKEN NMR center
Yokohama, Japan, October 21, 2006

(37) 'GFT projection NMR spectroscopy'
International Conference on Structural Genomics
Beijing, China, October 24, 2006

(38) 'Theory and Application of GFT NMR'
Beijing NMR center, Beijing University
Beijing, China, October 28, 2006

(39) 'Theory and Application of GFT NMR'
University of Science and Technology
Hefei, China, November 2, 2006

(40) 'Structural Genomics in an 'omics'-world'
University of Campinas
Campinas, Brazil, February 12, 2007

(41) 'GFT Projection NMR for high-throughput protein structure determination'
II Structural Biology Workshop of the LNLS
Campinas, Brazil, February 14, 2007

(42) 'GFT Projection NMR: Theory and Application'
Hong Kong University of Science and Technology
Hong Kong, March 5, 2007

(43) 'Structural genomics in an 'omics'-world'
SCAI of the Fraunhofer Gesellschaft
Bonn, Germany, May 7, 2007

(44) 'Where do we stand on projection NMR?'
Chianti Workshop
Vallombrosa, Italy, June 5, 2007

(45) 'Structural genomics in an 'omics'-world'
University of Florence
Florence, Italy, June 8, 2007

EXHIBIT 2

1. Szyperski, T. and Schwerdtfeger, P. (1989) On the Stability of Trioxo(h⁵-Cyclopentadienyl) Compounds of Manganese, Technetium and Rhenium: An *ab initio* SCF Study. *Angew. Chem. Int. Ed. Engl.* **28**, 1228-1231.
2. Neri, D., Szyperski, T., Otting, G., Senn, H. and Wüthrich, K. (1989) Stereospecific Nuclear Magnetic Resonance Assignments of the Methyl Groups of Valine and Leucine in the DNA-Binding Domain of the 434 Repressor by Biosynthetically Directed Fractional ¹³C Labeling. *Biochemistry* **28**, 7510-7516.
3. Szyperski, T., Neri, D., Leiting, B., Otting, G. and Wüthrich, K. (1992) Support of ¹H NMR Assignments In Proteins by Biosynthetically Directed Fractional ¹³C-labeling. *J. Biomol. NMR* **2**, 323-334.
4. Szyperski, T., Güntert, P., Otting, G. and Wüthrich, K. (1992) Determination of Scalar Coupling Constants by Inverse Fourier Transformation of In-Phase Multiplets. *J. Magn. Reson.* **99**, 552-560.
5. Szyperski, T., Güntert, P., Stone, S. R. and Wüthrich, K. (1992) Nuclear Magnetic Resonance Solution Structure of Hirudin(1-51) and Comparison with Corresponding Three-dimensional Structures Determined Using the Complete 65-Residue Hirudin Polypeptide Chain. *J. Mol. Biol.* **228**, 1193-1205.
6. Szyperski, T., Güntert, P., Stone, S. R., Tulinsky, A., Bode, W., Huber, R. and Wüthrich, K. (1992) Impact of Protein-Protein Contacts on the Conformation of Thrombin-bound Hirudin Studied by Comparison with the Nuclear Magnetic Resonance Solution Structure of Hirudin(1-51). *J. Mol. Biol.* **228**, 1206-1211.
7. Wüthrich, K., Szyperski, T., Leiting, B. and Otting, G. (1992) Biosynthetic Pathways of the Common Proteinogenic Amino Acids Investigated by Fractional ¹³C Labeling and NMR Spectroscopy. In: *Frontiers and New Horizons in Amino Acid research* (K. Takai, Ed.), Elsevier, Amsterdam, pp 41-48.
8. Szyperski, T., Wider, G., Bushweller, J. H. and Wüthrich, K. (1993) 3D ¹³C-¹⁵N Heteronuclear Two-spin Coherence Spectroscopy for Polypeptide Backbone Assignments in ¹³C-¹⁵N-double Labeled Proteins. *J. Biomol. NMR* **3**, 127-132.
9. Szyperski, T., Luginbühl, P., Otting, G., Güntert, P. and Wüthrich, K. (1993) Protein Dynamics studied by Rotating Frame ¹⁵N Spin Relaxation Times. *J. Biomol. NMR* **3**, 151-164.
10. Szyperski, T., Wider, G., Bushweller, J. H. and Wüthrich, K. (1993) Reduced Dimensionality in Triple Resonance Experiments. *J. Am. Chem. Soc.* **115**, 9307-9308.
11. Szyperski, T., Scheek, S., Johansson, J., Assmann, G., Seedorf, U. and Wüthrich, K. (1993) NMR determination of the Secondary Structure and the Three-dimensional Polypeptide Backbone Fold of the Human Sterol Carrier Protein 2. *FEBS Lett.* **335**, 18-26.
12. Johansson, J., Szyperski, T., Curstedt, T. and Wüthrich, K. (1994) The NMR Structure of the Pulmonary Surfactant-Associated Polypeptide SP-C in an Apolar Solvent Contains a Valyl-Rich α -Helix. *Biochemistry* **33**, 6015-6023.
13. Szyperski, T., Antuch, W., Schick, M., Betz, A., Stone, S. R. and Wüthrich, K. (1994) Transient Hydrogen Bonds Identified on the Surface of the NMR Solution Structure of Hirudin. *Biochemistry* **33**, 9303-9310.
14. Ottiger, M., Szyperski, T., Luginbühl, P., Ortenzi, C., Loporini, P., Bradshaw, R. A. and Wüthrich, K. (1994) The NMR Solution Structure of the Pheromone Er-2 From the Ciliated Protozoan *Euplotes raikovi*. *Protein Science* **3**, 1515-1526.
15. Szyperski, T., Pellecchia, M. and Wüthrich, K. (1994) 3D $H^{\alpha/\beta}C^{\alpha/\beta}(CO)NHN$, a Projected 4D NMR Experiment for the Sequential Correlation of Polypeptide

- $^1\text{H}^{\alpha/\beta}$, $^{13}\text{C}^{\alpha/\beta}$ and Backbone ^{15}N and $^1\text{H}^{\text{N}}$ Chemical Shifts. *J. Magn. Reson. B* **105**, 188-191.
16. Szyperski, T., Pellecchia, M., Wall, D., Georgopoulos, C. and Wüthrich, K. (1994) NMR Structure Determination of the *Escherichia coli* DnaJ Molecular Chaperone: Secondary Structure and Backbone Fold of the N-terminal Region 2-108 Comprising the Highly Conserved J-Domain. *Proc. Natl. Acad. Sci. USA* **91**, 11343-11347.
 17. Smith, P. E., van Schaik, R. C., Szyperski, T., Wüthrich, K. and van Gunsteren, W. F. (1995) Internal Mobility of the Basic Pancreatic Trypsin Inhibitor in Solution: A Comparison of NMR Spin Relaxation Measurements and Molecular Dynamics Simulations. *J. Mol. Biol.* **246**, 356-365.
 18. Johansson, J., Szyperski, T. and Wüthrich, K. (1995) Pulmonary Surfactant-Associated Polypeptide SP-C in Lipid Micelles: CD Studies of Intact SP-C and NMR Secondary Structure of Depalmitoyl-SP-C(1-17). *FEBS Lett.* **362**, 261-265.
 19. Szyperski, T., Braun, D., Fernández, C., Bartels, C. and Wüthrich, K. (1995) A Novel Reduced-Dimensionality Triple Resonance Experiment for Efficient Polypeptide Backbone Assignment, 3D COHNNCA. *J. Magn. Reson. B* **108**, 197-203.
 20. Szyperski, T. (1995) Biosynthetically Directed Fractional ^{13}C -labeling of Proteinogenic Amino Acids. An Efficient Analytical Tool to Investigate Intermediary Metabolism. *Eur. J. Biochem.* **232**, 433-448.
 21. Luginbühl, P., Szyperski, T. and Wüthrich, K. (1995) Statistical Basis for the Use of $^{13}\text{C}^{\alpha}$ Chemical Shifts in Protein Structure Determination. *J. Magn. Reson. B* **109**, 229-233.
 22. Zerbe, O., Szyperski, T., Ottiger, M. and Wüthrich, K. (1996) 3D ^1H -TOCSY-relayed ct- ^{13}C , ^1H]-HMQC for Aromatic Spin System Identification in Uniformly ^{13}C Labeled Proteins. *J. Biomol. NMR* **7**, 99-106.
 23. Pellecchia, M., Szyperski, T., Wall, D., Georgopoulos, C. and Wüthrich, K. (1996) NMR Structure of the J-domain and the Gly/Phe-rich Region of the *Escherichia Coli*. DnaJ Chaperone. *J. Mol. Biol.* **260**, 236-250.
 24. Szyperski, T., Braun, D., Banecki, B. and Wüthrich, K. (1996) Useful Information from Axial Peak Magnetization in Projected NMR Experiments. *J. Am. Chem. Soc.* **118**, 8147-8148.
 25. Szyperski, T., Bailey, J. E. and Wüthrich, K. (1996) Detecting and Dissecting Metabolic Fluxes Using Biosynthetic Fractional ^{13}C -labeling and Two-dimensional NMR Spectroscopy. *Trends in Biotechnology* **14**, 453-459.
 26. Fernández, C., Szyperski, T., Bruyère, T., Ramage, P., Mössinger, E. and Wüthrich, K. (1997) NMR Solution Structure of the Pathogenesis-Related Protein P14a. *J. Mol. Biol.* **266**, 576-593.
 27. Pellecchia, M., Iwai, H., Szyperski, T. and Wüthrich, K. (1997) The 2D NMR Experiments H(C)CO₂ and HCCO₂ for Assignment and pH Titration of Carboxylate Groups in Uniformly $^{15}\text{N}/^{13}\text{C}$ -Labeled Proteins. *J. Magn. Reson.* **124**, 274-278.
 28. Sauer, U., Hatzimanikatis, V., Bailey, J. E., Hochuli, M., *Szyperski, T. and Wüthrich, K. (1997) Metabolic Fluxes in Riboflavin-producing *Bacillus subtilis*. *Nature Biotechnol.* **15**, 448-452.
 29. Szyperski, T., Fernández, C. and Wüthrich, K. (1997) Two-dimensional ct-HC(C)H-COSY for Resonance Assignments of Smaller ^{13}C -labeled Biomolecules. *J. Magn. Reson.* **128**, 228-232.
 30. Szyperski, T., Ono, A., Fernández, C., Iwai, H., Tate, S., Wüthrich, K. and Kainosho, M. (1997) Measurement of $^3J_{\text{C}_2\text{P}}$ Scalar Couplings in a 17 kDa

- Protein Complex with ^{13}C , ^{15}N -Labeled DNA Distinguishes the B_I and B_{II} Phosphate Conformations of the DNA. *J. Am. Chem. Soc.* **119**, 9901-9902.
31. Klimasauskas, S., *Szyperski, T., Serva, S. and Wüthrich, K. (1998) Dynamic Modes of the Flipped-out Cytosine during *HhaI* Methyltransferase-DNA Interactions in Solution. *EMBO J.* **17**, 371-324.
 32. Szyperski, T., Fernández, C., Ono, A., Kainosho, M. and Wüthrich, K. (1998) Measurement of Deoxyribose $^3J_{\text{HH}}$ Scalar Couplings Reveals Protein-Binding Induced Changes in the Sugar Puckers of the DNA. *J. Am. Chem. Soc.* **120**, 821-822.
 33. Szyperski, T., Fernández, C., Mumenthaler, C. and Wüthrich, K. (1998) Structure Comparison of Human Glioma Pathogenesis-Related Protein GliPR and the Plant Pathogenesis-related Protein P14a Indicates a Functional Link between the Human Immune System and a Plant Defense System. *Proc. Natl. Acad. Sci. USA* **95**, 2262-2266.
 34. Szyperski, T., Banecki, B., Braun, D. and Glaser, R. W. (1998) Sequential Assignment of Medium-sized $^{15}\text{N}/^{13}\text{C}$ -labeled Proteins with Projected 4D Triple Resonance NMR Experiments. *J. Biomol. NMR* **11**, 387-405.
 35. Fernández, C., Szyperski, T., Ono, A., Iwai, H., Tate, S.-I., Kainosho, M. and Wüthrich, K. (1998) NMR with ^{13}C , ^{15}N -doubly-labeled DNA: the *Antennapedia* Homeodomain Complex with a 14mer DNA Duplex. *J. Biomol. NMR* **12**, 25-37.
 36. Szyperski, T. (1998) ^{13}C -NMR, MS and Metabolic flux Balancing in Biotechnology Research. *Q. Rev. Biophys.* **31**, 41-106.
 37. Weber, F. E., Dyer, J. H., López Garcia, F., Szyperski, T., Wüthrich, K. and Hauser, H. (1998) In Pre-sterol Carrier Protein 2 (SCP2) in Solution the Leader Peptide 1-20 is Flexibly Disordered and the Residues 21-143 Adopt the Same Globular Fold as in Mature SCP2. *Cell. Mol. Life Sci.* **54**, 751-759.
 38. Szyperski, T., Vandenbussche, G., Curstedt, T., Ruyschaert, J.-M., Wüthrich, K. and Johansson, J. (1998) Monomeric α -helical Pulmonary Surfactant-associated Polypeptide C Dissolved in a Mixed Organic Solvent Transforms Into Insoluble β -sheet Aggregates. *Protein Sci.* **7**, 2533-2540.
 39. Pervushin, K., Ono, A., Fernandez, C., Szyperski, T., Kainosho, M. and Wüthrich, K. (1998) NMR Scalar Couplings Across Watson-Crick Base Pair Hydrogen Bonds in DNA Observed by Transverse Relaxation-Optimized Spectroscopy. *Proc. Natl. Acad. Sci. USA* **95**, 14147-14151.
 40. Fiaux, J., Andersson, C. I. J., Holmberg, N., Bülow, L., Kallio, P. T., Szyperski, T., Bailey, J. E. and Wüthrich, K. (1999) ^{13}C NMR Flux Ratio Analysis of *Escherichia coli* Central Carbon Metabolism in Microaerobic Bioprocesses. *J. Am. Chem. Soc.* **121**, 1407-1408.
 41. Szyperski, T., Götte, M., Billeter, M., Perola, E., Cellai, L., Heumann, H. and Wüthrich, K. (1999). NMR Structure of r(gcacuggc)·r(gcca)d(CTGC), a Chimeric Hybrid Duplex Comprising the tRNA-DNA Junction Formed During the Initiation of HIV-1 Reverse Transcription. *J. Biomol. NMR* **13**, 343-355.
 42. Szyperski, T., Glaser, R. W., Hochuli, M., Fiaux, J., Sauer, U., Bailey, J. E. and Wüthrich, K. (1999) Bioreaction Network Topology and Metabolic Flux Ratio Analysis by Fractional ^{13}C -Labeling and Two-dimensional NMR Spectroscopy. *Metabolic. Eng.* **1**, 189-197.
 43. Hochuli, M., Patzelt, H., Österheld, D., Wüthrich, K. and *Szyperski, T. (1999) Amino Acid Metabolism in the Halophilic Archaeon *Haloarcula hispanica*. *J. Bacteriol.* **181**, 3226-3237.
 44. Szyperski, T., Fernandez, C., Ono, A., Wüthrich, K. and Kainosho, M. (1999) The $\{^{31}\text{P}\}$ -Spin-echo-difference Constant-time [^{13}C , ^1H]-HMQC Experiment for

- Simultaneous Determination of $^3J_{\text{H3'P}}$ and $^3J_{\text{C4'P}}$ in Nucleic Acids and their Protein Complexes. *J. Magn. Reson.* **140**, 491-494.
45. Fernandez, C., Szyperski, T., Billeter, M., Ono, A., Iwai, H., Kainosho, M. and Wüthrich, K. (1999) Conformational Changes of the BS2 Operator DNA upon Complex Formation with the *Antennapedia* Homeodomain Studied by NMR with $^{13}\text{C}/^{15}\text{N}$ -labeled DNA. *J. Mol. Biol.* **292**, 609-617.
 46. Sauer, U., Lasko, D. R., Fiaux, J., Hochuli, M., Glaser, R. W., Szyperski, T., Wüthrich, K. and Bailey, J. E. (1999) Metabolic Flux Ratio (METAFor) Analysis of Genetic and Environmental Modulations of *Escherichia coli* Central Carbon Metabolism. *J. Bacteriol.* **181**, 6679-6688.
 47. Lopez, F., Szyperski, T., Choinowski, T., Dyer, J. H., Hauser, H. and Wüthrich, K. (2000) NMR Structure of the Sterol Carrier Protein-2: Implications for the Biological Role. *J. Mol. Biol.* **295**, 595-603.
 48. Sauer, U., Szyperski, T. and Bailey, J. E. (2000) Future Trends in Complex Reaction Studies. In: *NMR in Microbiology: Theory and Application* (J.-N. Barbotin and J.-C. Portais, Eds.), Horizon Scientific Press, Norfolk.
 49. Skalicky, J. J. and Szyperski, T. (2000) Two-dimensional NMR. In: *NMR in Microbiology: Theory and Application* (J.-N. Barbotin and J.-C. Portais, Eds.), Horizon Scientific Press, Norfolk.
 50. Hochuli, M., Szyperski, T. and Wüthrich, K. (2000) Deuterium Isotope Effects on the Central Carbon Metabolism of *Escherichia coli* cells grown on a D_2O -containing Minimal Medium. *J. Biomol. NMR* **17**, 33-42.
 51. Skalicky, J. J., Sukumaran, D. K., Mills, J. L. and *Szyperski, T. (2000) Toward Structural Biology in Supercooled Water. *J. Am. Chem. Soc.* **122**, 3230-3231.
 52. Montelione, G. T., Zheng, D., Huang, Y., Gunsalus, K. C. and Szyperski, T. (2000) Protein NMR Spectroscopy for Structural Genomics. *Nature Struct. Biol.* **7**, 982-984.
 53. Frey, A. D., Fiaux, J., Szyperski, T., Bailey, J. E., Wüthrich, K. and Kallio, P. T. (2001) Dissection of the Central Carbon Metabolism of Hemoglobin-Expressing *Escherichia Coli* by ^{13}C NMR Flux Distribution Analysis in Microaerobic Bioprocesses. *Appl. Environ. Microbiol.* **67**, 680-687.
 54. Skalicky, J. J., Mills, J. L., Sharma, S. and *Szyperski, T. (2001) Aromatic Ring-flipping in Supercooled Water: Implications for NMR-based Structural Biology of Proteins. *J. Am. Chem. Soc.* **123**, 388-397.
 55. Canonaco, F., Hess, T. A., Wang, T., Szyperski, T. and Sauer, U. (2001) Metabolic Flux Response to Phosphoglucose Isomerase Knock-out in *Escherichia Coli*. *FEMS Microbiol. Lett.* **204**, 247-252.
 56. Maaheimo, H., Fiaux, J., Cakar, Z. P., Bailey, J. E., Sauer, U. and *Szyperski, T. (2001) Central Carbon Metabolism of *Saccharomyces cerevisiae* Explored by Biosynthetic Fractional ^{13}C Labeling of Common Amino Acids. *Eur. J. Biochem.* **268**, 2464-2479.
 57. Emmerling, M., Dauner, M., Ponti, A., Fiaux, J., Hochuli, M., Szyperski, T., Wüthrich, K., Bailey, J. E. and Sauer, U. (2001) Metabolic Flux Response to Pyruvate Kinase Knockout in *Escherichia Coli*. *J. Bacteriol.* **184**, 152-164.
 58. Dauner, M., Sonderegger, M., Hochuli, M., Szyperski, T., Wüthrich, K., Hohmann, H. P., Sauer, U. (2002) Metabolic Fluxes in Riboflavin-Producing *Bacillus Subtilis* During Growth on Two-carbon Substrate Mixtures. *Appl. Environ. Microbiol.* **68**, 1760-1771.
 59. Monleon, D., Colson, K., Moseley, H. N. B., Anklin, C., Oswald, R., Szyperski, T. and Montelione, G. T. (2002) Rapid Analysis of Protein Backbone Resonance Assignments using Cryogenic Probes, a Distributed Linux-based

- Computing Architecture, and an Integrated Set of Spectral Analysis Tools. *J. Struc. Func. Genomics* **2**, 93-101.
60. Szyperski, T., Yeh, D. C., Sukumaran, D. K., Moseley, H. N. B. and Montelione, G. T. (2002) Reduced-dimensionality NMR spectroscopy for High-Throughput Resonance Assignment. *Proc. Natl. Acad. Sci. USA* **99**, 8009-8014.
 61. Szymczynska, B. R., Pineda-Lucena, A., Mills, J. L., Szyperski, T. and Arrowsmith, C. (2002) ^1H , ^{13}C and ^{15}N resonance Assignments and Secondary Structure of the RNA-Binding PWI Domain from SRm160 using Reduced Dimensionality NMR Spectroscopy. *J. Biomol. NMR* **22**, 299-300.
 62. Mills, J. L. and *Szyperski, T. (2002) Protein Dynamics in Supercooled Water: The Search for Slow Motional Modes. *J. Biomol. NMR* **23**, 63-67.
 63. Szyperski, T. (2002) Strukturelle Genomik. *Nachrichten aus der Chemie* **50**, 1128-1131.
 64. Xia, Y., Arrowsmith, C. H. and *Szyperski, T. (2002) Novel Projected 4D Triple Resonance Experiments for Polypeptide Chemical Shift Assignment. *J. Biomol. NMR* **24**, 41-50.
 65. Gong, B., Zeng, H., Zhu, J., Yuan, L., Han, Y., Cheng, S., Furukawa, M., Parra, R. D., Kovalevsky, A. Y., Mills, J. L., Skrzypczak-Jankun, E., Martinovic, S., Smith, R. D., Zheng, C., Szyperski, T. and Zeng, X. C. (2002) Creating Nanocavities of Tunable Sizes: Hollow Helices. *Proc. Natl. Acad. Sci. USA* **99**, 11583-11588.
 66. Fiaux, J., Cakar, Z. P., Sonderegger, M., Wüthrich, K., *Szyperski, T. and Sauer, U. (2003) Metabolic Flux Profiling of the Yeasts *Saccharomyces cerevisiae* and *Pichia stipitis*. *Eucaryotic Cell* **2**, 170-180.
 67. Kim, S. and *Szyperski, T. (2003) GFT NMR, a New Approach to Rapidly Obtain Precise High Dimensional NMR Spectral Information. *J. Am. Chem. Soc.* **125**, 1385-1393.
 68. Daujotyte, D., Vilkaitis, G., Manelyt, L., Skalicky, J., *Szyperski, T. and Klimasauskas, S. (2003) Solubility Engineering *HhaI* Methyltransferase for NMR Structural Studies. *Protein Eng.* **16**, 295-301.
 69. Liu, G., Mills, J. L., Hess, T. A., Kim, S., Skalicky, J. J., Sukumaran, D. K., Kupce, E., Skerra, A., *Szyperski, T. (2003) Resonance Assignments for the 21 kDa Engineered Fluorescein-binding Lipocalin FluA. *J. Biomol. NMR* **27**, 187-188.
 70. Aramini, J. M., Mills, J. L., Xiao, R., Acton, T. B., Wu, M. J., Szyperski, T. and Montelione, G. T. (2003) Resonance Assignments for the Hypothetical Protein yggU from *Escherichia coli*. *J. Biomol. NMR* **27**, 285-286.
 71. Monleon, D., Chiang, Y., Aramini, J., Swapna, G.V.T., Palacios, D., Gunsalus, K.C., Kim, S., Szyperski, T. and Montelione, G. T. (2004) Resonance Assignments for the 21 kDa *Caenorhabditis elegans* Homologue of 'Brain-specific' Protein. *J. Biomol. NMR* **28**, 91-92.
 72. Kim, S. and *Szyperski, T. (2004) GFT Triple Resonance NMR Experiments for Polypeptide Chemical Shift Assignment. *J. Biomol. NMR* **28**, 117-130.
 73. Xu, D., Liu, G., Rong, X., Acton, T., Goldsmith-Fischman, S., Honig, B., Montelione, G. T. and *Szyperski, T. (2004) NMR Structure of the Hypothetical Protein AQ-1857 Encoded by the YI57 Gene from *Aquifex aeolicus* Reveals a Novel Protein Fold. *Proteins* **54**, 794-796.
 74. Liu, G., Sukumaran, D. K., Xu, D., Chiang, Y., Acton, T., Goldsmith-Fischman, S., Honig, B., Montelione, G. T. and *Szyperski, T. (2004) NMR Structure of the Hypothetical Protein NMA1147 from *Neisseria meningitidis* Reveals a Distinct 5-helix Bundle. *Proteins* **55**, 756-758.

75. Herve du Penhoat, C., Atreya, H. S., Shen, Y., Liu, G., Acton, T. B., Li, Z., Murray, D., Montelione, G. T. and *Szyperski, T. (2004) The NMR Solution Structure of the 30S Ribosomal Protein S27e Encoded in the Gene RS27_ARCFU of *Archaeoglobus fulgidis* Reveals a Novel Protein Fold. *Protein Sci.* **13**, 1407-1416.
76. Sola, A., Maaheimo, H., Ylonen, K., Ferrer, P. and *Szyperski, T. (2004) Amino Acid Biosynthesis and Metabolic Profiling of *Pichia pastoris*. *Eur. J. Biochem.* **271**, 2462-2470.
77. Zamboni, N., Maaheimo, H., Szyperski, T., Hohmann, H.-P. and Sauer, U. (2004) The Phosphoenolpyruvate Carboxykinase also Catalyzes C3 Carboxylation at the Interface of Glycolysis and the TCA Cycle of *Bacillus subtilis*. *Metabolic Eng.* **6**, 277-284.
78. Shen, Y., Atreya, H. S., Xiao, R., Acton, T. B., Shastri, R., Ma, L., Montelione, G. T. and *Szyperski, T. (2004) Resonance Assignment for the 18 kDa Protein CC1736 from *Caulobacter crescentus*, *J. Biomol. NMR* **29**, 549-550.
79. Moseley, H. N. B., Riaz, N., Aramini, J. M., Szyperski, T. and Montelione, G. T. (2004) A Generalized Approach to Automated NMR Peak List Editing: Application to Reduced Dimensionality Triple Resonance Spectra. *J. Magn. Reson.* **170**, 263-277.
80. Atreya, H. S. and *Szyperski, T. (2004) G-matrix Fourier Transform NMR Spectroscopy for Complete Protein Resonance Assignment. *Proc. Natl. Acad. Sci. USA* **101**, 9642-9647.
81. Yuan, L., Zeng, H., Yamato, K., Sanford, A. R., Feng, W., Atreya, H. S., Sukumaran, D. K., Szyperski, T. and Gong, B. (2004) Helical Aromatic Oligoamides: Reliable, Readily Predictable Folding from the Combination of Rigidified Structural Motifs. *J. Am. Chem. Soc.* **126**, 16528-16537.
82. Atreya, H. S. and *Szyperski, T. (2005) Rapid NMR Data Collection. *Methods Enzymol.* **394**, 78-108.
83. Huang, Y. J., Moseley, H., Baran, M. C., Arrowsmith, C. H., Powers, R., Tejero, R., Szyperski, T. and Montelione, G. T. (2005) An Integrated Platform for Automated Analysis of Protein NMR Structures. *Methods Enzymol.* **394**, 111-140.
84. Shen, Y., Goldsmith-Fischman, Atreya, H. S., Acton, T., Ma, L., Xiao, R., Honig, B., Montelione, G. T. and *Szyperski, T. (2005) NMR Structure of the 18 kDa Protein CC1736 From *Caulobacter crescentus* Identifies a Member of the 'START' Domain Superfamily and Suggests Residues Mediating Substrate Specificity. *Proteins* **58**, 747-750.
85. Liu, G., Li, Z., Chiang, Y., Acton, T., Montelione, G. T., Murray, D. and *Szyperski, T. (2005) High-quality Homology Models Derived From NMR and X-ray Structures of E. coli Proteins YgdK and SufE Suggest That All Members of the YgdK/SufE Protein Family are Enhancers of Cysteine Desulfurases. *Protein Sci.* **14**, 1597-1608.
86. Szyperski, T. (2005) Protein NMR Spectroscopy. In: *Encyclopedia of Molecular Cell Biology and Molecular Medicine*. Wiley-CH, Weinheim.
87. Herve du Penhoat, C., Li, Z., Atreya, H. S., Kin, S., Yee, A., Xiao, R., Murray, D., Arrowsmith, C. H. and *Szyperski, T. (2005) Solution NMR Structure of *Thermotoga maritima* Protein TM1509 Reveals a Zn-metalloprotease-like Tertiary Structure. *J. Struc. Func. Genomics* **6**, 51-62.
88. Atreya, H. S., Eletsky, A. and *Szyperski, T. (2005) Resonance Assignment of Proteins with High Shift Degeneracy Based on 5D Spectral Information Encoded in G²FT NMR Experiments. *J. Am. Chem. Soc.* **127**, 4554-4555.

89. Yang, S., Atreya, H. S., Liu, G. and *Szyperski, T. (2005) G-matrix Fourier Transform NOESY Based Protocol for High-Quality Protein Structure Determination. *J. Am. Chem. Soc.* **127**, 9085-9099.
90. Liu, G., Aramini, J., Atreya, H. S., Eletsky, A., Xiao, R., Acton, T. A., Ma, L. C., Montelione, G. T. and *Szyperski, T. (2005) GFT NMR Based Resonance Assignment for the 21 kDa Human Protein UFC1. *J. Biomol. NMR* **32**, 261.
91. Pineda-Lucena, A., Ho, C. S., Mao, D. Y., Sheng, Y., Laister, R. C., Muhandiram, R., Lu, Y., Seet, B. T., Katz, S., Szyperski, T., Penn, L. Z. and Arrowsmith, C. H. (2005) A Structure-based Model of the c-Myc/Bin1 Protein Interaction Shows Alternative Splicing of Bin1 and c-Myc Phosphorylation are Key Binding Determinants. *J. Mol. Biol.* **351**, 182-194.
92. Liu, G., Shen, Y., Atreya, H. S., Parish, D., Shao, Y., Sukumaran, D., Xiao, R., Yee, Adelinda, Lemak, A., Bhattacharya, A., Acton, T. A., Arrowsmith, C. H., Montelione, G. T. and *Szyperski, T. (2005) NMR Data Collection and Analysis Protocol for High-throughput Protein Structure Determination. *Proc. Natl. Acad. Sci. USA* **102**, 10487-10492.
93. Eletsky, A., Atreya, H. S., Liu, G. and *Szyperski, T. (2005) Probing Structure and Functional Dynamics of (large) Proteins with Aromatic Rings: L-GFT-TROSY (4,3)D ^1H - ^13C NMR Spectroscopy. *J. Am. Chem. Soc.* **127**, 14578-14579.
94. *Szyperski, T., Mills, J. L., Perl, D. and Balbach, J. (2006) Combined NMR-observation of Cold Denaturation in Supercooled Water and Heat Denaturation Enables Accurate Measurement of ΔC_p of Protein Unfolding. *Eur. Biophys. J.* **35**, 363-366.
95. Szyperski, T. (2006) Principles and Application of Projected Multidimensional NMR Spectroscopy – G-matrix Fourier Transform NMR. In: *Emerging Principles in Biophysics* (J.L.R. Arrondo and A. Alonso, Eds.), Springer Verlag, New York.
96. Liu, G., Shen, Y., Xiao, R., Acton, T. A., Ma, L. C., Joachimiak, A., Montelione, G. T. and *Szyperski, T. (2006) NMR Structure of Protein yqbG Encoded by Gene YQBG_BASCU From *Bacillus subtilis* Reveals a Novel α -Helical Protein Fold. *Proteins* **62**, 288-291.
97. *Szyperski, T. and Atreya, H. S. (2006) Principles and Applications of GFT Projection NMR Spectroscopy. *Magn. Reson. Chem.* **44**, 51-60.
98. Mukherjee, S., Muralidhar, D., Atreya, H. S., Szyperski, T., Jeromin, A., Sharma, Y. and Chary, K. V. (2006) ^1H , ^{13}C and ^{15}N Chemical Shift Assignments for Neuronal Calcium Sensor-1, a Multi-functional Calcium-binding Protein. *J. Biomol. NMR*, **36**, 48.
99. Lin, Y-C., Liu, G., Shen, Y., Bertonate, C., Yee, A., Honig, B., Arrowsmith, C., and *Szyperski, T. (2006) NMR Structure of Protein PA2021 From *Pseudomonas aeruginosa*. *Proteins*, **65**, 767-770.
100. Sola, A., Jouhten, P., Maaheimo, H., Sanchez-Ferrando, F., Szyperski, T. and Ferrer, P. (2007) Metabolic Flux Profiling of *Pichia pastoris* Grown on Glycerol/methanol Mixtures in Chemostat Cultures at Low and High Dilution Rates. *Microbiology*, **153**, 281-290.
101. Singarapu, K. K., Liu, G., Xiao, R., Bertonati, C., Honig, B., Montelione, G. T. and *Szyperski, T. (2007) NMR Structure of Protein yjbR from *Escherichia coli* Reveals 'Double-wing' DNA Binding Motif. *Proteins*, **67**, 501-504.
102. Atreya, H., Garcia, E., Shen, Y. and *Szyperski, T. (2007) J-GFT NMR for Precise Measurement of Mutually Correlated Spin-spin Couplings. *J. Am. Chem. Soc.*, **129**, 680-692.

103. Szyperski, T. (2007) On NMR-based Structural Proteomics. In: *Structural Proteomics* (Eds. J.L. Sussman and I. Silman), World Scientific Publishing, New York.
104. Shen, Y. and *Szyperski, T (2007) Structure of Protein BPTI Derived With NOESY in Supercooled Water: Validation and Refinement of Solution Structures. *Angew. Chem. Int. Ed. Engl.*, in press.
105. Singarapu, K.K., Xiao, R., Sukumaran, D. K., Acton, T., Montelione, G. T. and *Szyperski, T. (2007) NMR Structure of Protein Cgl2762 From *Corynebacterium glutamicum* Implicated in DNA Transposition Reveals a Helix-turn-helix Motif Attached to a Flexibly Disordered Leucine Zipper. *Proteins*, in press.
106. Singarapu, K.K., Xiao, R., Acton, T., Montelione, G. T. and *Szyperski, T. (2007) NMR Structure of the Peptidyl-tRNA Hydrolase Domain from *Pseudomonas syringae* Expands the Structural Coverage of the Hydrolysis Domains of Class 1 Peptide Chain Release Factors. *Proteins*, in press.